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(54) COMPOSITION HAVING LOCAL ANESTHETIC EFFECT

(71) We, ASTRA LAKEMEDEL AKTIEBOLAG, a Swedish Body Corporate, of Kvarnbergagatan 16, S-151 85 Sodertalje, Sweden, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to a local anesthetic composition which on topical application to unbroken skin gives a deeply penetrating local anesthetic effect with a sufficiently rapid onset and also a long duration after the removal of the composition from the site of application, to a method for the preparation of such a composition and to a method for improving the skin penetrating ability for a specific local anesthetic.

The efficacy of local anesthetics on the mucous membranes is well documented, but their effectiveness on the intact skin has not been established. For example it is known that some solutions and ointments containing the base form of some well-known local anesthetics may give a superficial local anesthetic effect on topical application to the unbroken skin (cf. Adriani et al: Anesthesia and Analgesia ... Vol. 50, No. 5, p. 834—40, 1971). However, the obtained effect was obviously weak and the blocking effect was transient and disappeared within 10 to 60 seconds after the test site was wiped dry. This lack of durable anesthesia *inter alia* means that these compositions would be useless for surgical purposes.

According to S. Monash (A.M.A. Archives of Dermatology, Vol. 76, p 752—56, Dec. 1957) it was possible to obtain lasting local anesthesia of unbroken skin with a reasonable time of onset (45 to 60 minutes) when using alcoholic solutions of the bases of lidocaine, tetracaine, phenacaine, benoxinate and tripelennamine. However, it has not been possible to verify these results of Monash with later and more sophisticated tests. This is probably due to the fact that the test method used by Monash involved a sort of continuous testing by pricking with a needle. If anesthesia was not apparent on testing at first test, the anesthetic composition was reapplied to the no longer intact skin whereupon renewed testing for anesthesia was undertaken. As pointed out by Adriani et al in the above mentioned publication (p. 834—35) such a test method is of doubtful value in assessing the efficacy of local anesthetics on application to unbroken skin.

In summary, before the date of the present invention there existed no local anesthetic composition which upon topical application to unbroken skin gave a deeply penetrating local anesthetic effect with a rapid onset and long duration of anesthesia after the removal of the composition from the site of application. It is obvious that such a composition would be useful for many applications of local anesthesia e.g. in minor surgery and in treatment of psoriasis or other skin diseases.

The present invention is based on the surprising finding that the ability of the base form of the local anesthetically active compound o-diisopropylaminoethoxybutyrophenone to penetrate unbroken skin is appreciably improved when said compound is dissolved in a solvent comprising 40—70% (v/v) isopropanol and 0—60% (v/v) glycerol.

Accordingly, the present invention provides to a local anesthetic composition having the ability to penetrate unbroken skin on topical application and giving a deep and prolonged anesthesia, which composition consists of a solution containing at least 1% (w/v) of the basic form of the compound o-diisopropylaminoethoxybutyrophenone dissolved in a solvent comprising 40—70% (v/v) isopropanol and 0—60% (v/v) glycerol, the balance, if any, being an inert constituent or a diluent not exceeding 40% of the total volume of solvent.

When the solvent contains inert constituents or diluents they may be e.g. water or viscosity increasing agents. Examples of suitable viscosity increasing agents are Carbopol resins (obtained from B. F. Goodrich Chemical Company) and pulverized silicone dioxide. Carbopol is a registered Trade Mark. The

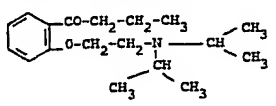
combined amount of these additional constituents or diluents may, however, not exceed 40% of the total volume of the solvent.

A preferred composition according to the invention will be obtained if the solvent consists of 60—70% (v/v) isopropanol and 0—40% (v/v) glycerol. A further preferred solvent consists of 60—70% (v/v) isopropanol and 20—40% (v/v) glycerol. An especially effective composition will be obtained if the solvent consists of 60—70% (v/v) isopropanol, 20—40% (v/v) glycerol and 5—20% (v/v) water (the designations w/v and v/v used within this specification represent weight (g)/volume (ml) and volume/volume, respectively).

Results from pharmacological tests indicates that the depth and duration of anesthesia are affected in a negative way if the isopropanol content of the solvent exceeds 70% (v/v). It has also been found that the solvent should contain at least 40% (v/v) isopropanol. Further, the presence of at least 10% (v/v) glycerol is desirable to avoid irritating effects of the composition according to the invention.

It can be noted that solvents containing isopropanol and glycerol have previously been used for dissolving local anesthetics. However, there is nothing in the prior art which indicates that the ability of a specific local anesthetic to penetrate unbroken skin might be appreciably improved by dissolving it in a solvent containing certain specified concentrations of isopropanol and glycerol. Nor is there any indication in the prior art that the possible skin penetrating ability of the compound o-diisopropylaminoethoxybutyrophenone might be appreciably improved by the application of such a solvent. In fact the penetrating ability of the composition according to the invention is sufficiently high to give detectable quantities of o-diisopropylaminoethoxybutyrophenone in the blood upon topical application to intact skin of humans. In this connection it can be mentioned that Adriani et al could not find detectable quantities of local anesthetic agents in the blood following topical application of known local anesthetic preparations to normal skin (cf. page 838 of the above mentioned publication of Adriani et al).

The composition according to the invention should preferably contain 5—15% (w/v) of the basic form of the compound o-diisopropylaminoethoxybutyrophenone. Further, the composition should preferably not contain more of said compound than can be completely dissolved in the applied solvent at 0°C. This compound has the formula



and the properties of the hydrochloride of this compound are described in U.S. Patent No. 3,335,184. Also a method for the preparation of this compound is described in said patent. The method involves condensation of o-hydroxybutyrophenone with diisopropylamino-ethylchloride hydrochloride in alcoholic medium in the presence of 2 equivalents of sodium methanolate. The base which forms is isolated and precipitated with HCl in ethanol from a solution in benzene. The base form can be isolated from the hydrochloride according to methods known *per se*. Any medical use for the base form of the compound o - diisopropylaminoethoxybutyophenone has not to our knowledge been proposed before the date of our invention. The pharmacological tests described in the above mentioned U.S. Patent No. 3,335,184 are only concerned with the properties of the corresponding hydrochloride, and it is a well-known fact that the local anesthetic properties of bases and salts are usually widely different, *inter alia* due to different lipid solubilities.

The present invention also includes a method for improving the skin penetrating ability of the basic form of the compound o-diisopropylaminoethoxybutyrophenone, by dissolving the compound in a solvent comprising 40—70% (v/v) isopropanol and 0—60% (v/v) glycerol, the balance if any being an inert constituent or a diluent not exceeding 40% of the total volume of solvent.

In clinical practice the composition according to the invention is applied topically to the skin in such amounts and during such times that are necessary for obtaining the desired local anesthetic effect. The site of application can optionally be covered with a dressing, such as an occlusive dressing, after the application of the composition.

It is to be understood that the composition according to the invention can also be used for conventional surface anesthesia such as anesthesia of open wounds, mucous membranes etc. according to methods known to the art of surface anesthesia. Following the strong penetrating ability of the composition according to the invention it is in these cases usually quite sufficient if the composition contains between 1—5% (w/v) of o-diisopropylaminoethoxybutyrophenone. The same consideration applies when the composition according to the invention is used for anesthesia of unbroken but not really intact skin, e.g. for anesthesia of burned or sunburned skin and for anesthesia in connection with various skin diseases.

The composition according to the invention can be prepared by any appropriate mixing operation. This is exemplified in the following Example:

Example:

100 g of dry o-diisopropylaminoethoxybutyrophenone (base) is transferred to a vessel at room temperature. A solvent containing isopropanol, glycerol and water is prepared by mixing 650 ml isopropanol, 250 ml glycerol, and 100 ml water at room temperature in a separate vessel. This solvent is slowly added to the first vessel while stirring. The addition is stopped when enough solvent has been added to give 1000 ml of the final composition. The resulting composition contains 10% (w/v) of o-diisopropylaminoethoxybutyrophenone in the form of a clear, viscous solution which is stable and can be stored at a temperature between 0—30°C.

The surprising local anesthetic activity of the composition according to the invention can be demonstrated by the following pharmacological tests.

Pharmacological tests

Guinea-pigs were shaved or treated with hair remover (e.g. Opilca®). The skin, which now was smooth and hairless, was washed with 0.1% Desivon®.

A gauze folded twice (23 mm diameter) was put into the test solution and thereafter in a plastic cup (outer diameter 26 mm, inner diameter 24 mm, inner height 4 mm).

The cup with the gauze saturated with test solution was attached to the back of the animal as an occlusive dressing fastened with plaster (25 mm width) wound around the body. The time of contact was registered. After the end of the contact time the site of contact was washed with 0.1% Desivon® and marked with a fatty chalk. With a sharp instrument (e.g. cannula or a pair of tweezers) the place was pricked six times and registration was made as in the intracutaneous wheal test (Bülbring — Wajda: J. Pharmacol., 1945, *85*, p 78) on the back of the guinea-pig, i.e. by registering the presence or absence of a characteristic ripple of the skin of the back of the animal. Every pricking which gives no skin contraction was counted. The test solution was applied to groups of four animals. The anesthetic effect was tested by pin-pricking 6 times within the treated area at regular intervals of time after the end of the application period. Complete anesthesia accordingly corresponds to 24 counts. The degree of anesthesia was calculated as the quotient

$$\frac{(\text{count}) \times 100}{24}(\%)$$

The results from tests with various compositions of o-diisopropylaminoethoxybutyrophenone in the appropriate solvent are shown in Table 1. The amount of o-diisopropylaminoethoxybutyrophenone was in all tests one part by weight (g) dissolved to ten parts by volume (ml) by addition of the solvent, i.e. the concentration was 10% (w/v). The time of application was 30 minutes.

TABLE 1

Topical anesthesia of unbroken guinea-pig skin with o-diisopropylaminoethoxybutyrophenone base (10 % w./v) dissolved in various vehicles (Application period - 30 minutes)

Solvent (v/v %)			Local anesthetic effect	
Isopropyl alcohol	Glycerol	Water	Degree (%)	Duration (min) of complete anesthesia
60	40	0	100	10
60	25	15	100	50
60	20	20	100	30
65	35	0	100	30
65	25	10	100	50
65	10	25	100	60 1)
65	5	30	100	90 1)
65	0	35	100	55 1)
70	30	0	75	–
70	20	10	88	–
70	10	20	100	25 1)
80	20	0	21	–
90	10	0	67	–

1) slight irritation of the skin for at least one tested animal

As can be seen from Table 1 the results indicate an anesthetic effect of sufficient duration to be of clinical value.

It should be noted that the above described valuable results are specific for the particular combination of vehicle and local anesthetic agent used within this invention and that unsatisfactory results are obtained if o-diisopropylaminoethoxybutyrophenone base is replaced by some other local anesthetic. This is illustrated in Table 2 below which shows the effects of o-diisopropylaminoethoxybutyrophenone base (10% w/v), tetracaine base (10% w/v), benzocaine base (10% w/v) and lidocaine base (20% w/v) dissolved in a solvent consisting of 65% (v/v) isopropanol, 25% (v/v) glycerol and 10% (v/v) water.

TABLE 2

Topical anesthesia of unbroken guinea-pig skin with some local anesthetics (base) dissolved in isopropanol (65 %, v./v) glycerol (25 %, v./v) and water (10 %, v./v) (Application period = 30 minutes)

Agent	Conc. (% w./v)	Local anesthetic effect: Degree (%)	Duration (min) of complete anasthesia	Duration (min) when the effect had subsided to 50%	Complete recovery (min)
1. o-Diisopropyl aminoethoxy-butyrophenone	10	100	55	155	230
2. Lidocaine (Xylocaine®)	20	92	–	10	20
3. Tetracaine (Pontocaine)	10	8	–	–	10
4. Benzocaine	10	0	–	–	–

As can be seen from Table 2 the solutions of tetracaine and benzocaine had no significant effect. The effect with double the concentration of lidocaine was very weak and of short duration. In contrast diisopropylaminoethoxybutyrophenone base gave rise to complete local anesthesia of long duration. When using the composition containing this latter compound it was also possible to make deep incisions in the treated area without the animals showing signs of pain.

It should be noted that all of the above described tests have been performed with solutions of local anesthetic bases. The corresponding local anesthetic salts e.g. the hydrochlorides are less effective for anesthesia of unbroken skin irrespective of the particular solvent used. E.g. when using a composition of o - diisopropylaminoethoxybutyrophenone hydrochloride (10% w/v) dissolved in a solvent containing 65% (v/v) isopropyl alcohol, 25% (v/v) glycerol and 10% (v/v) water in a guinea-pig test as described above it was not possible to obtain complete anesthesia even after an application period of 60 minutes.

WHAT WE CLAIM IS:—

1. A local anesthetic composition having the ability to penetrate unbroken skin on topical application and giving a deep and prolonged anesthesia comprising a solution containing at least 1% (w/v) of o-diisopropylaminoethoxybutyrophenone in basic form dissolved in a solvent comprising 40—70% (v/v) isopropanol and 0—60% (v/v) glycerol, the balance, if any, being an inert constituent or a diluent not exceeding 40% of the total volume of solvent.

2. A composition according to claim 1 wherein the solvent comprises 60—70% (v/v) isopropanol and 0—40% (v/v) glycerol.

3. A composition according to claim 1 wherein the solvent comprises 60—70% (v/v) isopropanol and 20—40% (v/v) glycerol.

4. A composition according to claim 1 wherein the solvent comprises 60—70% (v/v) isopropanol, 20—40% (v/v) glycerol and 5—20% (v/v) water.

5. A composition according to any one of the preceding claims wherein the solution contains 5—15% (w/v) of o-diisopropylaminoethoxybutyrophenone.

6. A composition according to any one of the preceding claims wherein the concentration of o-diisopropylaminoethoxybutyrophenone in the solvent does not exceed the solubility of o-diisopropylaminoethoxybutyrophenone in the solvent at 0°C.

7. A composition according to claim 1 substantially as hereinbefore described.

8. A process for the preparation of a composition according to claim 1 which comprises incorporating in a solvent as defined in claim 1 an amount of o-diisopropylaminoethoxybutyrophenone in basic form such that the final composition will contain at least 1% (w/v) of o-diisopropylaminoethoxybutyrophenone.

9. A method for improving the skin penetrating ability of o-diisopropylaminoethoxybutyrophenone in basic form by dissolving at least 1% (w/v) o-diisopropylaminoethoxybutyrophenone in a solvent comprising

40—70% (v/v) isopropanol and 0—60% (v/v) glycerol, the balance, if any, being an inert constituent or a diluent not exceeding 40% of the total volume of solvent.

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(19)

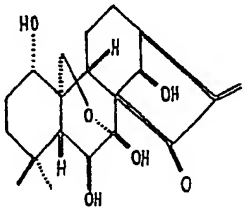
(54) PHARMACEUTICAL COMPOSITIONS HAVING ANTI-TUMOR ACTIVITY

(71) We, NIPPON SHINYAKU COMPANY LIMITED, of 14 Kisshoin Nishinosho Monguchicho, Minami-ku, Kyoto, Japan, a Company organised and existing under the laws of Japan, do hereby declare the invention, for which we pray that a Patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

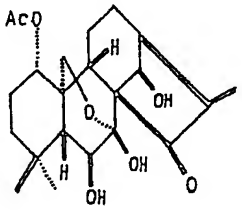
The present invention relates to pharmaceutical compositions having anti-tumor activity.

Accordingly the present invention is a pharmaceutical composition suitable for use as an anti-tumor agent which comprises oridonin and/or lasiokaurin and a solid or liquid carrier substance.

Oridonin and lasiokaurin are diterpenoids isolated from the *Isodon* plants of Labiatae, and their structures are shown as follows:—



Oridonin



Lasiokaurin

Oridonin was first isolated from the leaves of *Isodon japonicus* (Japanese name: Hikiokoshi) growing wild in Kochi district (Japan) and was shown to have m.p. 248—250° C (decompn) and $[\alpha]_D$-46°. Lasiokaurin was obtained from *Isodon lasiocarpus* (Japanese name: Taiwan-hikiokoshi) growing in Formosa and has m.p. 228—229° C and $[\alpha]_D^{17}$-94°. The structures and absolute configurations of these compounds have been elucidated by Fujita, one of the inventors, and his co-workers. See, for instance: E. Fujita, *et al., J. Chem. Soc (C)*, 1970, 1674—1681 and E. Fujita *et al., Chem. Pharm. Bull.* (Tokyo) *20*, 1752—1754 (1970).

The plants, *Isodon japonicus* and *I. trichocarpus* containing the oridonin or lasiokaurin in compounded or impure form, have been used as the bitter peptic in the home remedy, and reputed to be of marvellous efficacy for stomachache as well as gastrointestinal disorders. The investigation for the effective components, however, had little developed.

According to the present invention it has been found unexpectedly that the foregoing oridonin and lasiokaurin had a remarkable anti-tumor activity. The pharmacological experimental data of oridonin and lasiokaurin for anti-tumor activity are shown below.

The anti-tumor activity was assessed by injection of a solution of oridonin or lasiokaurin in 20% ethanol into the test animals. The male mice of ddY-strain (average body weight: 20±0.5 g) were used. Erlich ascites cell was inoculated into peritoneam of the mice, and the said tumor cells were adjusted to 2×10^6 cells/mouse. After 24 hours from inoculation, 0.25 ml of the foregoing solution or oridonin or laskiokaurin was injected intraperitoneally. Injection was repeated 7 times every 24

hours. These mice were observed for 40 days and the anti-tumor activity of oridonin and lasiokaurin was judged by comparison to the numbers of the dead mice with those of the control. The results are shown in the Table 1. The numerals in the table show percentage of the survival.

TABLE 1

Materials Given and Their Doses	Oridonin				Lasiokaurin	
Days Elapsed	5	10	15 mg./kg	Control	10 mg./kg	Control
1	100%	100%	100%	100%	100%	100%
2	100	100	100	100	100	100
3	100	100	100	100	100	100
4	100	100	100	100	100	100
5	100	100	100	100	100	100
6	100	100	100	100	100	100
7	100	100	100	100	100	100
8	100	100	100	100	100	100
9	100	100	100	100	100	100
10	100	100	100	100	100	100
11	100	100	100	100	100	100
12	100	100	100	100	100	100
13	100	100	100	100	100	100
14	100	100	100	100	100	100
15	100	100	100	90	100	80
16	80	100	100	80	100	80
17	60	100	100	60	100	70
18	60	100	100	40	100	60
19	50	90	100	30	80	50
20	30	90	100	10	80	20
21	20	80	100	0	80	0
22	0	70	90	0	70	0
23	0	70	90	0	70	0
24	0	70	90	0	70	0
25	0	70	80	0	70	0
26	0	60	80	0	70	0
27	0	60	80	0	70	0
28	0	60	80	0	50	0
29	0	60	80	0	50	0
30	0	60	80	0	50	0
31	0	60	80	0	50	0
32	0	60	80	0	50	0
33	0	60	70	0	50	0
34	0	60	70	0	50	0
35	0	60	70	0	50	0
36	0	60	70	0	40	0
37	0	60	70	0	40	0
38	0	60	70	0	40	0
39	0	60	70	0	40	0
40	0	60	70	0	40	0
Mean Survival Days	18.0	32.4	35.5	17.1	30.4	17.6 days

As clarified by the foregoing experimental results, the dosage of 10 mg/kg and of 15 mg/kg of oridonin to mice showed the effect for survival time for 15.3 and 18.6 days on the average, respectively. Similarly, the dosage of 10 mg/kg of kasiokaurin to mice was effective for the prolongation of life for 12.8 days on the average. On the other hand, the experiments for the acute toxocity to mice (by intraperitoneal injection) gave 35—40 mg/kg as the LD_{50} value for oridonin and more than 70 mg/kg for lasiokaurin. These values are much larger than the foregoing effective doses. Oridonin and lasiokaurin are, therefore, safe and effective anti-tumor agents.

For the human body, oral administration, injection or other routes can be used. In the case of oral administration, the compounds are used as powders or tablets mixed with non-toxic carriers, e.g. milk sugar (lactose) starch (dextrin) etc. or as solution or emulsion. The dose may change depending on the condition and age of the patient.

These compounds dissolved in water, ethyl alcohol or mixtures of alcohol and water are used also as injection. In this case, some solubilizer may be added at need. The solution thus prepared, the patient is dosed by means of an intraperitoneal or intravenous injection. The dosage may change in compliance with the condition and age of the patient.

This invention, thus, provides the new and useful anti-tumor agents which show an excellent effect for the prolongation of survival in the Ehrlich ascites tumor-inoculated mice and are expected to be effective for several other tumors including cancer.

WHAT WE CLAIM IS:—

1. A pharmaceutical composition suitable for use as an anti-tumor agent which comprises oridonin and/or lasiokaurin and a solid or liquid carrier substance.

2. A pharmaceutical composition as claimed in Claim 1 wherein the solid or liquid carrier substance is selected from milk sugar (lactose) starch (dextrin), water, ethyl alcohol and mixtures thereof.

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